Protocols for
Concentration Gradient Experiment

1. Prepare the protein solutions

All the proteins were stored in the 20mM TBS (pH=7.3), and the concentration of specific protein was previously detected by Thermo® NANODROP 2000 Spectrophotometer. For experimental use, those solutions are diluted to about 1mg/mL, 5mg/mL and 10mg/mL by certain volume of 20mM TBS (pH=7.3). The final concentration of protein solutions are shown below:

Protein Contained	Mw (kDa)	Mass Concentration	Molar Concentration	Mass Concentration	Molar Concentration	Mass Concentration	Molar Concentration
3A-SUP	21.4	1.018mg/mL	47.59μM	5.090mg/mL	237.95μM	10.18mg/mL	475.9μM
3A-mSA	25.4	1.209mg/mL	47.59μM	6.045mg/mL	237.95μM	—*	—*
3B	55.4	1.000mg/mL	18.05μM	5.000mg/mL	90.25	10.00mg/mL	180.5μM

*: The highest concentration of 3A-mSA is lower than 10mg/mL. So no sample containing 10mg/mL 3A-mSA was prepared.

2. Establish the reaction system

Three experiments same to each other were made to ensure parallelity.

The volume of reaction system must be greater than 200μL, and the ratio between monomers containing A and 3B was 5.5:14.5, which is the result of obeying the rule that the quantity of A and B must be approximately equal to each other. Particular pH (pH=7.3) and particular temperature (T=25C°) were introduced to perform the concentration gradient experiment.

Reactants were firstly placed on heaters for 5 minutes to ensure they had reached the reactive temperature (25C°), and then the reactants were mixed according to their pH and the reactions began. During the reaction, 20μL samples were extracted every 30 minutes and the whole process lasted for 2 hours.

3. Post-processing

Before being added to the PCR tubes, samples containing 5mg/mL and 10mg/mL should be diluted to 1mg/mL by certain volume of TBS (pH=7.3). Then the diluted samples should be added to the tubes.

All the PCR tubes prepared to load samples were added in advance with 7.5μL 5x protein loading buffer and 10μL TBS. Then 20 μL samples were added into the tubes and then boiled at 95C° for 7 minutes. After the boiling process, samples were placed at 25C° for 10 minutes to ensure every sample were cooled, and then centrifugation was introduced to shake down the condensate water in the purpose of keeping the volume. Then, adding 7.50μL for reactive samples and 5.00μL for Markers to the wells and running SDS-PAGE for a certain time at 150V was required, followed by Coomassie Brilliant Blue staining process.

After the process, SDS-PAGE gels were scanned and analyzed by software lane 1D to find out the surplus content of monomers (must be with negative control groups) and the mass distribution of oligomers. Results were further analyzed to be charted or graphed.